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Growth and morphological responses of *Andrographis paniculata* to varying shade and nitrogen fertilization

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Abstract

Andrographis paniculata (Burm. f.) Nees is a traditional medicinal plant with valuable phytochemical and pharmacological potential. Growth and morphological responses to light and N can be useful measurements to determine favorable growing conditions for *A. paniculata*. Despite numerous findings on other medicinal and aromatic plants, there is little information about how light and N affect growth and morphology *A. paniculata*. The objective of this study was to determine the effects of shade and N on growth and morphological responses of *A. paniculata*. Plants were grown under two shade levels, 0% and 40%, and fertilized with five N rates, 90, 135, 180, 225 and 270kg ha^{-1} in a nested design. Shaded plants grew taller with greater total leaf area, specific leaf area, leaf area ratio and net assimilation rate than sun-grown plants. Fertilizing plants with increasing rate of N has increased their height, leaf area index, total leaf area, shoot and root dry mass, leaf mass ratio and root shoot ratio. There was a quadratic relationship between N rate and total dry mass of plants. The goal in commercial *A. paniculata* cultivation is to produce high yielding high quality plants. Results showed that *A. paniculata* could adapt to varying levels of shade and N by altering its growth and morphology. Shading at 40% and fertilizing with 225kg N ha^{-1} can increase growth and yield of *A. paniculata*.

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Introduction

Light and nitrogen (N) are important environmental factors affecting plant growth and yield potential (Chapin *et al.* 1987; Singhal *et al.* 1999). Plants can respond to variation in light and N availability by changing their morphology, and anatomy such as developing higher leaf and cuticle thickness (Briskin and Gawienowski 2001; Casey *et al.* 2004), adjusting leaf surface and canopy size (Volkenburgh 1999; Knops and Reinhart 2000; Oikawa *et al* 2005). They can partition assimilated carbons among sink organs (Poorter and Nagel 2000). As a result, plants can lower their root shoot ratio (Gedroc *et al* 1996; Weiner 2004), enhance leaf mass ratio and net assimilation rate. This phenotypic plasticity is one of the ways plants adapt to changes in their environment and to light and nutrient stress (Whitman and Agrawal 2009; Gratani 2014).

Besides nutrients, light is the most limiting growth factor. The structural organization of plants relies on the extent, bearing, period and quality of light (Bjorn 2008). Light is the driving force for photosynthesis. Studies suggest that N redistribution both within and between leaves is a mechanism for photosynthetic acclimation to the current light environment. Nitrogen is one of the most expensive nutrients to supply. Commercial fertilizers represent the major cost in crop production (FAO 2011). Since N is a part of chlorophyll and nucleic acid, growth reduces when N in soil is not optimal (Barker and Pilbeam 2007; Ehsanipour *et al.* 2012). In addition, N loss in the field is becoming a cause of serious concern, giving rise to soil and water pollution. Improving crop yield correlates with applying optimum level of light and N. Some common strategy farmers use to enhance yield and quality of crops is by growing them under varying levels of shade and applying different rates of N fertilizers. Reports show that plants respond positively to light, and N fertilization (Pitono *et al.* 1996; Zaharah *et al.* 2001; Palaniswamy 2005; Suryawati *et al.* 2007; Kumar *et al.* 2008; Saravanan *et al.* 2008; Kumar *et al.* 2012), and N fertilization (Chauhan *et al.* 2002; Chen 2004; Singh and Singh 2004; Parvin 2007; Ambarwati 2008; Tiwari *et al.* 2012).

Andrographis paniculata (Burm. f.) Nees is a popular traditional herb in Malaysia. In recent years, *A. paniculata* has received much attention because of its medicinal properties and other uses that offer tremendous economic benefits. The plant has valuable phytochemicals such as terpenoids, phenolics and flavonoids. Researchers conduct many studies to test the phytochemical and pharmacological potential of *A. paniculata*. Studies show that *A. paniculata* can fight and prevent many chronic diseases such as cancer, atherosclerosis, diabetes, bronchitis, influenza, rheumatoid arthritis, ulcer, cardiovascular diseases, malaria and inflammation (Ramlan *et al.* 2005; Khatun *et al.* 2011; Subramaniam *et al.* 2012). Although *A. paniculata* is renowned for its medicinal properties, researchers are also exploring the possibility of applying this plant in various fields such as forestry, animal production or manufacturing (Valdiani *et al.* 2012). Consistent supply of raw herbal materials is vital to sustain these efforts. To meet the exploding global market, attempts are being made to cultivate *A. paniculata* on a commercial scale (Rahman 2012). Researchers are studying the agronomic requirements to enhance the growth and quality of *A. paniculata* (Ramesh *et al.* 2011; Mishra and Jain 2013).

Studies have reported that light and N can affect growth and morphology of *A. paniculata*. Plants grown under shaded conditions were taller with greater leaf, stem and root dry mass (Pitono *et al.* 1996), and developed larger surface leaf area (Palaniswamy 2005) than sun-grown plants. But a study also found that growth and yield were similar between shaded and sun-grown plants (Zaharah *et al.* 2002). Compared to light, N affects growth more than photosynthesis. Growth and yield of *A. paniculata* increases when fertilized with increasing rates of N, studies show (Sanjutha *et al.* 2008). But applying more than 557.7mg N per 3kg soil reduced total dry mass and relative growth rate of plants (Singh and Singh 2004). In another study, applying 45kg ha^{-1} manure and 60kg N ha^{-1} produced the tallest *A. paniculata* with the greatest number of leaves and total dry matter. But this combination of cow dung and N produced the lowest root dry matter (Parvin 2007).

Another study found *A. paniculata* increased its total dry mass when fertilized with 200kg N ha^{-1} (Ambarwati 2008). In addition, fertilizing with 60kg N ha^{-1} can increase leaf area and leaf area index (LAI) of *A. paniculata* (Tiwari *et al.* 2012).

Understanding how *A. paniculata* responds to light and N may improve the management practices under different conditions. Although findings are available on the effects of light and N on *A. paniculata*, the information is lacking. This study aims to find out whether varying levels of shade and N affects growth and morphological response of *A. paniculata*.

Materials and methods

Plant Materials and Seedling Establishment

This study involved growing and sampling *A. paniculata* plants accessioned from Perak, Malaysia with the accession number 11265. This accession was selected because the plants have superior agronomic traits than other *A. paniculata* accessions (Abdalla 2005). Seeds were obtained from Agro Gene Bank, Faculty of Agriculture, Universiti Putra Malaysia. Studies have reported that *A. paniculata* seeds are difficult to germinate because of inherent dormancy (Chauhan *et al.* 2009; Gagare and Mate 2009; Talei *et al.* 2012; Wong *et al* 2015). To break dormancy, one thousand seeds were first soaked in hot water at 50°C for three minutes (Saraswathy *et al.* 2004). Seeds were then sown between two sheets of filter paper moistened with distilled water in disposable Petri dishes at room temperature (24°C). Seven days after sowing, individual newly emerged seedlings were carefully selected using clean forceps and planted into moistened Jiffy pots (Jiffy-7® peat pellets, Hummert TM International, Earth City, MO).

The Jiffy pots were arranged in rectangular plastic trays. The trays were placed and arranged on a bench under a rain shelter at Farm 2, Universiti Putra Malaysia. To provide extra shading for seedlings, the trays were covered with a layer of green 50% shade nets. Seedlings were irrigated manually by pouring the same volume of distilled water into each tray to remoisten the peat pots. Irrigation frequency was adjusted depending on weather and stage of seedling growth.

At 14 and 28 days after sowing, water-soluble foliar fertilizer, Welgro® (15:30:15) was sprayed onto seedlings in each tray at 2g L^{-1} using a two-liter pressurized hand sprayer. Each spraying lasted about five minutes with the same nozzle setting and spray distance.

Before transplanting, potting media were prepared by thoroughly mixing sieved topsoil, peat and sand (2:1:1 v/v) in a concrete mixer. The finished potting mix was then poured into black polyethylene planting bags, 12cm diameter x 14cm high, in equal volumes and labeled according to treatments. Seedlings were hardened off by gradually placing them under full sunlight seven days before transplanting. Three hundred sixty healthy seedlings were selected and transplanted to the planting bags. The seedlings were one month old and averaged 5cm tall with at least four true leaves based on random sampling of 30 seedlings. Upon transplanting, the Jiffy-7 peat was left intact while the mesh surrounding the pellet was removed.

Experimental Design and Treatments

A pot experiment was conducted in a shade house (N 3°32' E 101°42') and open field (N 101°34' E 101°42') at Farm 2, Universiti Putra Malaysia. Plants were grown under two shade levels, 0% (full sunlight) and 40%, and fertilized with five N rates, 90, 135, 180, 225 and 270kg N ha^{-1} in a nested design. Nitrogen rates were replicated six times, nested within each shade level and arranged in randomized complete block design. Shade and N treatments were immediately initiated on the day of transplanting. The shade structure consisted of a wooden frame covered with commercial 40% black shade cloth. Shading percentage was tested by measuring the photosynthetic photon flux (PPFD) density in the shade structure and outdoor environment with a quantum sensor (LI-250A, LI-COR, Lincoln, Nebraska, USA), daily for a week on both sunny and overcast days. Under the shade structure, daytime PPFD averaged between 790 and 1100μmol m^{-2} s^{-1} while outdoor readings averaged between 1340 and 1860μmol m^{-2} s^{-1}. Nitrogen was side-dressed by hand along with phosphorus (P) and potassium (K). Sources of nutrients were urea, triple superphosphate (P_2O_5) and muriate of potash (K_2O).

Nitrogen was side-dressed at the five treatment rates while phosphorus and potassium were both side-dressed at 180kg ha^{-1}. Nitrogen, phosphorus and potassium were split-applied into three equal doses, with the first dose side-dressed on the day of transplanting, followed by a second and final dose respectively side-dressed 14 and 28 days after transplanting (DAT).

Agronomic and Cultural Management

Plants were irrigated using automatic sprinkler system set at both experimental sites. Irrigation frequency was adjusted depending on weather and stage of plant growth. On sunny days, the sprinkler system was typically set to irrigate the plants at 0800, 1200 and 1700h for 15 minutes each time. To eliminate weed growth, black plastic mulch was placed under the outdoor planting bags. Weeds growing on the surface of potting media were hand pulled as they emerged and discarded.

Plant Height and Canopy Measurement

Plant height was recorded at 14, 28, 42 and 56 days after transplanting while canopy was measured at 28 and 55 days after transplanting using nondestructive technique. Both plant height and canopy were measured on the same plants. Plant height was measured from the base of the plant at media surface to the top of the youngest fully expanded leaf using a steel meter-ruler (Cornelissen 2003; Perez-Harguindeguy *et al*. 2013). Canopy, expressed as leaf area index (LAI) is the total one-sided area of leaf tissue per unit ground surface area (Breda 2003). Leaf area index was measured using a plant canopy analyzer (LAI-200, LI-COR, Lincoln, Nebraska, USA) by a sequence of one-above and four-below canopy readings from each plant side, between 0800 and 1100h on a sunny day. Leaf area index readings between two and five were obtained from 60 plants at each sampling time (Malone *et al*. 2002).

Leaf, Stem and Root Dry Mass Measurement

Plants were destructively harvested 14, 28, 42 and 56 days after transplanting. On each harvest, 60 plants were harvested, with 20 plants per replicate per shade and N treatment. Therefore, 300 plants were destructively harvested which include 240 treated plants, and 60 seedlings. During each harvesting, whole plants were carefully uprooted from wet soil retaining even the fine roots. Plants were thoroughly washed free of soil particles using tap water and blotted dry with towels. Plants were then kept in plastic bags with holes, labeled according to treatment and immediately brought to the laboratory. Plants were carefully separated into leaves with petioles intact, stems and roots. Plant parts were put into separate pre-weighed brown A4 envelopes. The envelopes were labeled according to plant part and treatment. Plant parts were dried at 70°C for 48h in forced-draught stainless-steel-lined ovens (Memmert, Germany), with the envelopes top flaps left opened (Reuter and Robinson 1997). Dry mass of each plant part minus the envelopes was weighed (Sartorius, Germany).

Total Leaf Area Measurement

Before measuring the dry mass of plant parts, a leaf area meter (LI-3100C, LI-COR, Lincoln, Nebraska, USA) was used to measure total leaf area per plant. Leaves of each plant with petioles intact were gently blotted dry with paper towels. Individual leaves of each plant were then placed diagonally one after another between the guides on the moving lower transparent belt. The leaves on the moving belt were allowed to pass through the leaf area meter. As each leaf passed under the light source, the meter automatically totaled the accumulating leaf area. Total leaf area per plant was recorded after each sample reading was completed.

Growth Analysis

Sixty uniform healthy seedlings were randomly selected before initiating shade and nitrogen treatments at 0 days after transplanting. The seedlings were measured for initial height, total leaf area and plant dry mass following the methods described previously. Total leaf area and dry mass measurements were used to calculate indices of plant growth and allocation according to the classical approach (Hunt 1982, 1991, 2003). In the classical approach, basic growth variables were calculated across one harvest-interval, which is the period between two successive harvests.

In this study, the indices measured were total plant dry mass, total shoot dry mass, leaf area ratio (LAR), specific leaf area (SLA), root shoot ratio (RSR), leaf mass ratio (LMR), relative growth rate (RGR) and net assimilation rate (NAR). The initial mass to calculate growth, relative growth rate, and net assimilation rate were the leaf, stem and root dry mass averaged over the 60 randomly selected seedlings at 0 days after transplanting.

Statistical Analysis

For each growth variable, data were analyzed using statistical software package (SAS version 9.2, SAS Institute Incorporated, Cary, North Carolina, USA) by the general linear model (PROC GLM). Shade and N were the main fixed factors with N nested within shade. When F values were significant ($P<0.05$), means were separated with Fisher's protected least significant difference test ($P<0.05$). Where necessary, data were transformed to meet the assumptions of normality and homoscedasticity. Regression models were developed and evaluated on total dry mass and shoot dry mass at 56 days after transplanting. Curves were fitted by the least square method using a software (Sigma Plot version 11.0 for Windows, Systat Software Incorporated, Chicago, Illinois, USA).

Results

Plant Height and Canopy

Table 1 shows that shade significantly affected ($P<0.05$) plant height at all sampling times. Plants were between 50 and 58cm tall for both shade and N treatments at 56 DAT. Previous study found that sun-grown *A. paniculata* from different accessions were between 46 and 62cm tall, which is almost similar to the current finding (Prathanturarug *et al.* 2007). At 56 DAT, shaded plants were 7% taller than sun-grown plants. At 14, 28 and 42 DAT, shaded plants were also taller than sun-grown plants respectively by 35%, 28% and 30%. Previous study reported that *A. paniculata* grown under 50% shade were taller than sun-grown plants (Saravanan *et al.* 2008). Table 1 also shows that N significantly affected ($P<0.05$) plant height at 14, 42 and 56 DAT. Plants grew tall when fertilized with increasing rates of N. At 56 DAT, plants fertilized with 225kg N ha^{-1} were 15% taller than plants fertilized with 90kg N ha^{-1}. However, applying N beyond 225kg ha^{-1} did not cause difference ($P>0.05$) in the height of plants.

Table 1. Effects of shade and nitrogen (N) on plant height and leaf area index (LAI) of *A. paniculata* at different sampling times. Different letters are significantly different based on analysis of variance and Fisher's protected least significant difference means separation test ($P<0.05$).

	Days after transplanting					
	14	28	42	56	28	55
	Height (cm $plant^{-1}$)				LAI	
Shade (%)						
0 (full sunlight)	7.1^{b}	20.0^{b}	24.4^{b}	51.3^{b}	2.5^{a}	3.5^{a}
40	9.6^{a}		31.8^{a}	54.9^{a}	3.1^{a}	4.1^{a}
N Rate (kg ha^{-1})						
90	7.3^{b}	22.3^{a}	26.6^{b}	50.0^{b}	2.4^{b}	3.4^{b}
135	7.6^{b}	22.5^{a}	27.3^{b}	49.5^{b}	2.8^{a}	3.8^{a}
180	8.7^{ab}	21.5^{a}	27.1^{b}	50.2^{b}	2.9^{a}	3.9^{a}
225	9.7^{a}	23.0^{a}	28.2^{b}	57.4^{a}	3.0^{a}	4.0^{a}
270	8.5^{ab}	24.5^{a}	31.0^{a}	57.9^{a}	2.9^{a}	3.9^{a}
F value	***	***	***	*	ns	ns
Shade	*		**	***	*	*
N		ns	ns			

The height of plants interrelates with their canopy (Falster and Westoby 2003). Table 1 shows that shade did not affect ($P>0.05$) LAI of plants at 28 and 55 DAT. Previous study also found LAI between shaded and sun-grown *A. paniculata* did not differ (Zahara *et al.* 2001). Although statistically not significant, shaded plants respectively had 24% and 17% greater LAI than sun-grown plants at 28 and 55 DAT. Table 4.1 shows that N significantly affected ($P<0.05$) LAI of plants at 28 and 55 DAT.

Leaf area index ranged 2.4 to 4.0. At 28 DAT, plants fertilized with 135 kg N ha^{-1} had 17% greater LAI, than plants fertilized with 90kg N ha^{-1}. However, applying N beyond 135kg ha^{-1} did not cause difference (P>0.05) in LAI. At 55 DAT, plants fertilized with 135kg N ha^{-1} had 12% greater LAI than plants fertilized with 90kg N ha^{-1} at 28 DAT. However, applying N beyond 135kg ha^{-1} did not cause difference (P>0.05) in LAI of plants. Previous study found fertilizing with 120kg N ha^{-1} significantly increased LAI of Greek oregano (Sotiropoulo and Karamanos 2010), which is almost similar to current finding.

Leaf Characteristics

Table 3 shows that shade did not affect (P>0.05) total leaf area per plant of *A. paniculata* at all sampling times. Although not statistically significant, at 42 and 56 DAT, shaded plants respectively had 36% and 35% larger leaf area than sun-grown plants. Previous study found that total leaf area per plant of shaded *A. paniculata* was the highest while sun-grown plants produced the lowest leaf area (Palaniswamy, 2005).

Table 2. Effects of shade and nitrogen (N) on total leaf area and leaf dry mass of *A. paniculata* at different sampling times. Different letters are significantly different based on analysis of variance and Fisher's protected least significant difference means separation test (P<0.05).

	Days after transplanting							
	14	28	42	56	14	28	42	56
	Total leaf area (cm^2 plant^{-1})				Leaf dry mass (g plant^{-1})			
Shade (%)								
0	207.3^a	343.8^a	904.0^a	1427.8^a	1.0^a	1.5^a	6.7^a	13.5^a
40	281.1^a	372.7^a	1225.4^a	1924.5^a	1.1^a	1.2^a	5.5^a	12.7^a
N Rate (kg ha^{-1})								
90	224.4^a	332.1^a	807.1^d	1003.1^c	1.0^a	1.1^a	4.4^c	8.1^c
135	233.0^a	371.7^a	872.9^{cd}	1502.2^b	1.0^a	1.4^a	5.1^c	11.4^b
180	257.4^a	375.8^a	1028.3^{bc}	1602.0^b	1.1^a	1.5^a	6.3^b	12.9^b
225	246.6^a	370.8^a	1208.0^{ab}	2034.9^a	1.1^a	1.5^a	7.3^{ab}	16.0^a
270	257.7^a	340.9^a	1355.9^a	2217.8^a	1.1^a	1.3^a	8.0^a	17.0^a
F value								
Shade	ns	ns	ns	ns	ns	ns	ns	ns
N	ns	ns	**	**	ns	ns	**	**

After the previous sampling at 28 DAT, total leaf area per plant in shaded plants has increased by 229% while in sun-grown plants, total leaf area has increased by 229%. Table 3 also shows that N significantly affected (P<0.05) total leaf area per plant of *A. paniculata* at 42 and 56 DAT. Fertilizing plants with increasing rates of N significantly increased total leaf area at 42 DAT and 56 DAT. An earlier study also found that increasing the rates of N enhanced the total leaf area of *A. paniculata* (Tiwari *et al.* 2012). At 42 DAT, total leaf area ranged 807.1 to 1355.9cm^2 per plant. Plants fertilized with 225kg N ha^{-1} developed 38% greater leaf area than those fertilized with 180 kg N ha^{-1}. In addition, plants fertilized with 225kg N ha^{-1} produced 50% greater leaf area than plants fertilized with 90kg N ha^{-1}. However, fertilizing plants with N beyond 225kg ha^{-1} did cause difference in leaf area.

At 56 DAT, total leaf area ranged 1003.1 to 2217.8cm^2 per plant. Plants fertilized with 225kg N ha^{-1} developed 27% greater leaf area than plants fertilized with 180kg N ha^{-1}. In addition, plants fertilized with 225kg N ha^{-1} produced 103% greater leaf area than plants fertilized with 90kg N ha^{-1}. However, fertilizing plants with N beyond 225kg ha^{-1} did not cause difference in leaf area. Fig. 4.10a shows there was a significant interaction (P<0.05) between shade and N treatments on total leaf area of *A. paniculata* at 56 DAT. Shaded plants had greater leaf area than sun-grown plants when fertilized with increasing rates of N.

Leaf area closely relates with its dry mass. Table 3 shows that shade did not affect (P>0.05) leaf dry mass per plant of *A. paniculata* at all sampling times.

Although not statistically significant, at 42 and 56 DAT, sun-grown plants respectively had 22% and 6% larger leaf area than shaded plants. Previous study found that the total leaf area per plant of shaded *A. paniculata* was the highest while sun-grown plants produced the lowest leaf area (Saravanan *et al.* 2008). After the previous sampling at 28 DAT, leaf dry mass per plant in sun-grown plants has increased by 347% while in shaded plants, total leaf area has increased by 358%. Table 3 also shows that N significantly affected (P<0.05) leaf dry mass per plant of *A. paniculata* at 42 and 56 DAT. Fertilizing plants with increasing rates of N significantly increased leaf dry mass at 42 DAT and 56 DAT. An earlier study also found that increasing the rates of N enhanced the total leaf area of *A. paniculata* (Tiwari *et al.* 2012). At 42 DAT, leaf dry mass ranged 4.4 to 8.0g per plant. Plants fertilized with 225kg N ha^{-1} had 16% more leaf dry mass than those fertilized with 180 kg N ha^{-1}. In addition, plants fertilized with 225kg N ha^{-1} had 66% more leaf dry mass than plants fertilized with 90kg N ha^{-1}. However, fertilizing plants with N beyond 225kg ha^{-1} did not cause difference in leaf dry mass. At 56 DAT, leaf dry mass ranged 8.0 to 17g per plant. Plants fertilized with 225kg N ha^{-1} produced 24% greater leaf dry mass than plants fertilized with 180 kg N ha^{-1}. In addition, plants fertilized with 225kg N ha^{-1} produced 100% greater leaf dry mass than plants fertilized with 90kg N ha^{-1}. However, fertilizing plants with N beyond 225kg ha^{-1} did not cause difference in leaf dry mass.

Table 3. Effects of shade and nitrogen (N) on specific leaf area (SLA) and leaf area ratio (LAR) of *A. paniculata* at different sampling times. Different letters are significantly different based on analysis of variance and Fisher's protected least significant difference means separation test (P<0.05).

	Days after transplanting							
	14	28	42	56	14	28	42	56
	SLA (cm^2 g^{-1})				LAR (cm^2 g^{-1})			
Shade (%)								
0	230.4[a]	234.7[a]	133.7[b]	107.7[b]	149.2[a]	149.7[b]	83.7[b]	51.5[b]
40	274.0[a]	366.2[a]	230.2[a]	152.5[a]	167.6[a]	187.1[a]	142.5[a]	76.6[a]
N Rate (kg ha^{-1})								
90	248.0[a]	377.5[a]	183.7[a]	127.8[a]	153.8[a]	168.8[a]	107.3[a]	63.1[a]
135	250.7[a]	284.8[a]	185.6[a]	132.6[a]	161.0[a]	168.3[a]	117.9[a]	65.7[a]
180	242.0[a]	256.9[a]	178.2[a]	126.1[a]	145.1[a]	158.9[a]	111.9[a]	63.4[a]
225	251.8[a]	251.7[a]	180.4[a]	130.6[a]	161.7[a]	165.2[a]	114.0[a]	62.4[a]
270	265.6[a]	322.5[a]	173.4[a]	133.4[a]	168.4[a]	179.6[a]	109.1[a]	65.8[a]
F value								
Shade	ns	ns	**	**	ns	*	**	**
N	ns	ns	ns	ns	ns	ns	ns	ns

Table 3 shows that, at 42 and 56 DAT, shade significantly affected (P<0.05) SLA of *A. paniculata*. At 42 and 56 DAT, shaded plants respectively had 72% and 42% greater SLA than sun-grown plants. After the previous sampling at 28 DAT, SLA in shaded plants has decreased by 37% while in sun-grown plants, total leaf area has decreased by 43%. Shade did not affect (P>0.05) SLA of *A. paniculata* at 14 and 28 DAT. Table 3 also shows that, at 28, 42 and 56 DAT, shade significantly affected (P<0.05) LAR of *A. paniculata*. At 28, 42 and 56 DAT, shaded plants respectively had 25%, 70% and 49% greater SLA than sun-grown plants. After the previous sampling at 28 DAT, LAR in shaded plants has decreased by 24% while in sun-grown plants, LAR has decreased by 44%. Shade did not affect (P>0.05) SLA of *A. paniculata* at 14 and 28 DAT. Table 3 also shows that N did not affect (P>0.05) SLA and LAR of *A. paniculata* at all sampling times.

Dry Mass Partitioning

Results show that dry mass of *A. paniculata* increased with increasing age. However, shade and N did not affect (P>0.05) dry mass of plants at 14 and 28 DAT. Plants started responding to shade and N treatment at 42 and 56 DAT. Fig. 1 shows that shade significantly affected (P<0.05) root dry mass of plants at 42 DAT.

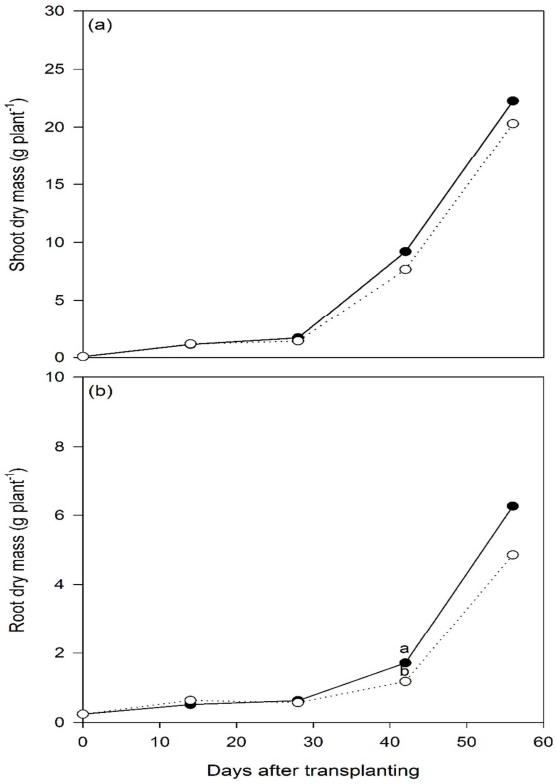

Fig. 1. Effects of shade on (a) shoot and (b) root dry mass of *A. paniculata* at 0, 14, 28, 42 and 56 days after transplanting. Plants were grown under 0% shade (●) and 40% shade (○).

Shaded plants produced 45% greater root dry mass than sun-grown plants. However, shade did not affect root dry mass at 14, 28 and 56 DAT. Fig. 1 also shows that shade did not affect ($P>0.05$) shoot dry mass at all sampling times. Although statistically not significant, shoot dry mass of shaded plants was slightly higher than sun-grown plants. Fig. 2 shows that, at 42 and 56 DAT, N significantly affected ($P<0.05$) shoot dry mass of *A. paniculata*. At 42 DAT, plants fertilized with 225kg N ha^{-1} produced 66% and 83% greater shoot dry mass than plants with 135 and 90kg N ha^{-1} respectively. Plants also responded similarly to N at 56 DAT. Plants fertilized with 225kg N ha^{-1} produced about 100% greater shoot dry mass than plants fertilized with 90 kg N ha^{-1}. Plants fertilized with 225kg N ha^{-1} also produced 25% greater shoot dry mass than plants fertilized with 180kg N ha^{-1}. At 56 DAT, plants produced more root dry mass when fertilized with N above 180kg ha^{-1}.

Plants fertilized with 225kg N ha^{-1} had 85% and 58% greater root dry mass than plants fertilized with 90kg N ha^{-1} and 180kg N ha^{-1} respectively. However, fertilizing with N beyond 225kg ha^{-1} did not cause a difference for both shoot and root dry mass. Fig. 3 shows a significant quadratic relationship between N rate and total dry mass of *A. paniculata* at 56 DAT. The equation describing the model is total dry mass = $2.17 + 0.18N - 0.0002N^2$ ($R^2 = 0.5$). There was also a quadratic relationship between N rate and shoot dry mass at 56 DAT. The equation describing the model is shoot dry mass = $-0.15 + 0.16N - 0.0002N^2$ ($R^2 = 0.5$). Both these data indicate that fertilizing with N beyond 225kg ha^{-1} will not increase total dry mass or shoot dry mass of plants.

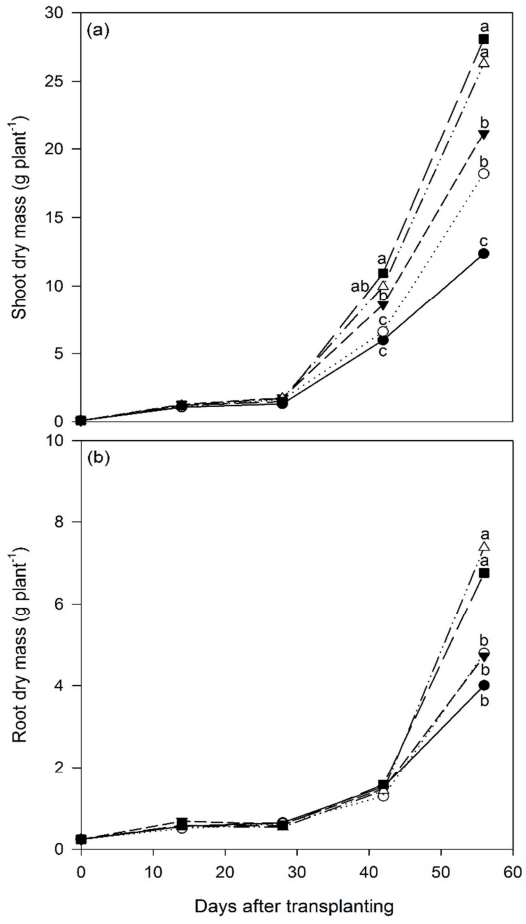

Fig. 2. Effects of nitrogen on (a) shoot and (b) root dry mass of *A. paniculata* at 0, 14, 28, 42 and 56 days after transplanting. Plants were fertilized with 90 (●), 135 (○), 180 (▼), 225 (△) and 270 (■) kg N ha^{-1}.

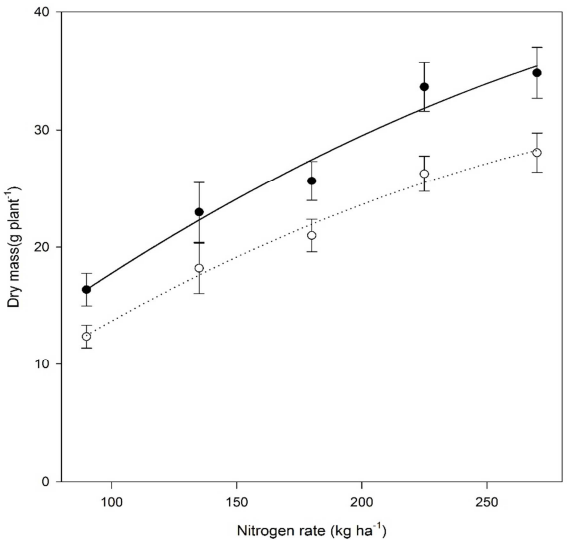

Fig. 3. Relationship between nitrogen rate and total dry mass (●), and shoot dry mass (○) of *A. paniculata* after final harvest or 56 days after transplanting. The equations are total dry mass = 2.17 + 0.18N - 0.0002N², R^2 = 0.5, and shoot dry mass = -0.15 + 0.16N - 0.0002N², R^2 = 0.5.

Table 4 shows shade did not affect (P>0.05) LMR and RSR of *A. paniculata* at all sampling times. After previous sampling at 42 DAT, LMR of shaded plants has decreased by 19% while in sun-grown plants, LMR has decreased by 24%. Root shoot ratio of shaded and sun-grown plants has also respectively reduced by 55% and 49% after the sampling at 28 DAT. This indicated that plants had adjusted the way they partitioned dry mass between roots and shoots. Table 4.4 shows N did not affect LMR at 14, 28 and 56 DAT. However, N significantly affected (P<0.05) LMR at 42 DAT. Plants fertilized with 135kg N ha^{-1} had 8.5% greater LMR than plants fertilized with 90kg N ha^{-1}. Applying N beyond 135kg ha^{-1} did not cause a difference in LMR of plants. Increased LMR has also increased RSR of plants fertilized with low N rates.

Table 4. Effects of shade and nitrogen (N) leaf mass ratio (LMR) and root shoot ratio (RSR) of *A. paniculata* at different sampling times. Different letters are significantly different based on analysis of variance and Fisher's protected least significant difference means separation test (P<0.05).

	Days after transplanting							
	14	28	42	56	14	28	42	56
	LMR (cm^2 $plant^{-1}$)				RSR (g g^{-1})			
Shade (%)								
0	0.64^a	0.64^a	0.63^a	0.48^a	0.44^a	0.39^a	0.20^a	0.28^a
40	0.60^a	0.60^a	0.62^a	0.50^a	0.52^a	0.44^a	0.20^a	0.26^a
N Rate (kg ha^{-1})								
90	0.61^a	0.57^a	0.59^b	0.49^a	0.51^a	0.55^a	0.28^a	0.32^a
135	0.63^a	0.62^a	0.64^a	0.50^a	0.45^a	0.42^a	0.21^b	0.27^{ab}
180	0.60^a	0.65^a	0.63^a	0.50^a	0.54^a	0.37^a	0.21^b	0.23^b
225	0.63^a	0.66^a	0.64^a	0.48^a	0.44^a	0.32^a	0.16^{bc}	0.28^{ab}
270	0.63^a	0.62^a	0.63^a	0.49^a	0.46^a	0.43^a	0.16^c	0.24^b
F value	ns	ns	ns	ns	ns	ns	ns	ns
Shade	ns	ns	**	ns	ns	ns	***	*
N								

Relative Growth Rate and Net Assimilation Rate

Fig. 4 shows that shade did not affect (P>0.05) RGR at all sampling times. Shade also did not affect NAR at 14, 28 and 56 DAT. However, shade significantly affected (P<0.05) NAR at 42 DAT.

Shaded plants had 60% greater NAR than sun-grown plants. This indicated that, at 42 DAT, shade leaves were more efficient and produced greater dry mass than sun leaves at 42 DAT. Fig. 5 shows that N did not affect (P>0.05) RGR at all sampling times. Nitrogen also did not affect NAR at 14, 28 and 42 DAT.

However, N significantly affected (P<0.05) NAR at 56 DAT. Plants fertilized with 135kg N ha^{-1} had 79% higher NAR than plants fertilized with 90kg N ha^{-1}. However, fertilizing with N beyond 135kg ha^{-1} did not cause a difference in NAR of plants.

Table 4 shows N significantly affected (P<0.05) RSR of plants at 42 and 56 DAT. Plants fertilized with 90kg N ha^{-1} had 33% greater RSR than plants fertilized with the highest rate of N at 270kg N ha^{-1}.

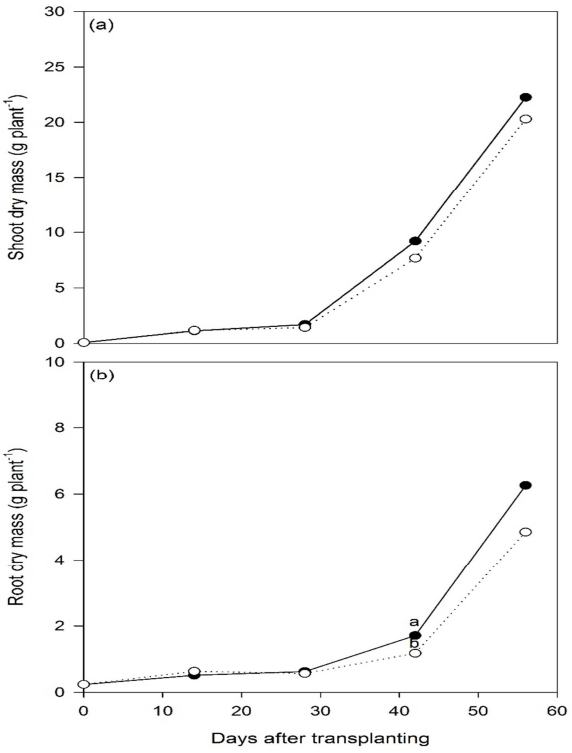

Fig. 4. Effects of shade on relative growth rate and net assimilation rate of *A. paniculata*. Plants were grown under 0% shade (●) and 40% shade (○).

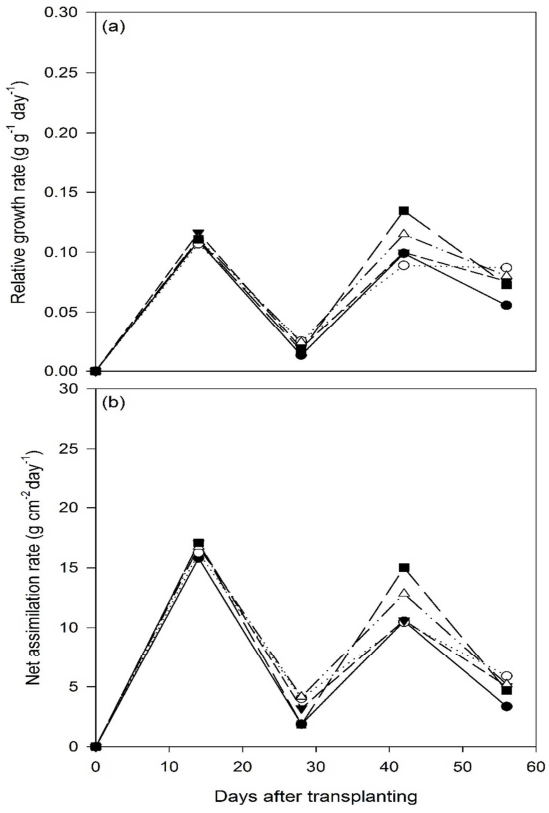

Fig. 5. Effects of shade on relative growth rate and net assimilation rate of *A. paniculata*. Plants were grown under 0% shade (●) and 40% shade (○).

Discussion

Plants react to both biotic and abiotic stimuli in different ways. Since plants are still organisms, they have no chance to escape the changing environmental conditions and attacks from other organisms. They must use other strategies to protect themselves against these situations (Lambers *et al.* 2008). Plasticity allows a plant to survive and adapt in a challenging landscape. Phenotypic plasticity is the ability of a genotype to produce different phenotypes from responding to distinct environmental conditions (Pigliucci 1997; 2005). All plasticity is physiological, but plants can manifest this by modifying its biochemistry, morphology, behavior, or life history (Sultan 2000, 2010). Light controls plant growth and development, and it affects them in a complex way at all phases of growth (Bjorn 2008). Nitrogen is another primary factor limiting plant production in many terrestrial ecosystems (Elberse *et al.* 2003). Plants minimize the effects of limiting light or nutrient by changing their growth characteristics and morphology (Reynolds and D' Antonio 1996; Poorter and Nagel 2000).

Height and canopy determine the morphology of a plant, which relates to its strategy, climatic factors and land use (Heady 1957; Moles *et al.* 2009). Plant height is central to a species' strategy to gain carbon because height determines the plant's ability to compete for light. Plant height correlates with traits such as LMR, LAR, leaf N per area, leaf mass per area and canopy area. These traits are essential in determining how plants live, grow and reproduce (Falster and Westoby 2003; Moles *et al.* 2009). Plant canopy structure is the spatial arrangement of the aboveground organs of plants in a plant community (Campbell and Norman 1990). Leaf area index is the total one-sided area of leaf tissue per unit ground surface area indicating the leafiness of a plant (Watson 1947). Leaf area index drives both the within and the below canopy microclimate which determines and controls, how much the canopy intercepts light, water and carbon gas exchange. Changes in LAI either by frost, storm, defoliation, drought or management practice, modify plant productivity (Beadle 1997; Breda 2003).

Under natural conditions, *A. paniculata* grows in both shaded and open areas. They also thrive in different types of soil at varying levels of fertility (Zaharah *et al.* 2006). Results indicate that *A. paniculata* is sensitive to variation in light and N availability. The findings so far are consistent with the optimal partitioning theory, which states that plants prefer to allocate resource to the organ that acquires the most limiting resource (McCarthy and Enquist 2007). Shaded *A. paniculata* grew taller with larger canopy than those sun-grown to optimize their capacity to capture light. This phenomenon is a typical example of the shade avoidance syndrome (Bou-Torrent 2008; Ruberti *et al.* 2012). Plants grew tall because their stems elongate from responding to red to far-red ratio the phytochrome perceived. Red to far-red ratio is the ratio of light at 655 to 665nm and at 725 to 735nm. Red light suppresses stem from elongating while far-red light enhances it (Smith 2000; Franklin and Whitelam 2005). Shade decreases this ratio by stimulating the cells of stems to make more phytohormones such as auxin, cytokinin and gibberellin. These phytohormones cause the stems to elongate resulting in tall plants. Studies are also showing that phytohormone activity may interact with N signaling to alter the physiology and morphology of plants (Morelli and Ruberti 2002, Friml 2003; Kozuka *et al.* 2010; Kiba *et al.* 2011; Tanimoto 2012). The same mechanism may drive the canopy plasticity of plants. This might explain why shaded *A. paniculata* increased their height and canopy when more N was available. Theories suggest that plant height depends on stem elongation while the canopy identifies leaf area (Russell *et al.* 1990; Falster and Westoby 2003).

Leaf area is a crucial variable for studying primary production in plants. Like LAI, measuring surface leaf area also indicates the productivity of plants (Inze *et al.* 2012). Leaf area and its mass affect SLA and LAR. Results showed that by increasing the rate of N, *A. paniculata* increased its leaf size. Cell division and cell elongation are the key processes driving the growth of leaves (Gonzales *et al.* 2012). When cells divide and elongate, they require more resources such as water and nutrient, triggering plants to increase uptake of these resources (Beadle 1997). Nitrogen is a constituent of all amino acids, proteins and enzymes. By increasing the rates of N, plants can use the surplus N to increase their leaf size. Small thick leaves usually develop in the sun while large thin leaves in the shade for many plants. Leaves must be wide and as flat and thin as possible to absorb sufficient light energy and facilitate gas exchange (Terashima *et al.* 2001; Tsukaya 2005; Valladares and Niinemets 2008). Results showed that shaded hempedu bumi had greater specific leaf area and leaf area ratio than sun-grown ones. This indicated that shaded hempedu bumi enhanced their capacity to capture light by producing thin leaves with high surface leaf area but low dry mass. Thin shade leaves might also have less palisade cells in the mesophyll which increases the path length of light (Vogellman *et al.* 1996; Terashima and Yano 2001). This strategy is also consistent with the optimal partitioning theory (McCarthy and Enquist 2007; Kobe *et al.* 2010). Leaves are the most dominant photosynthetic organ regulating dry mass production. This study showed that *A. paniculata* adjusted the size and shape of their leaves when grown under varying levels of shade and N. When leaf size and structure changes, it can also affect how plants distribute the dry mass between support and functional tissues.

Dry mass partitioning is the fraction of total dry mass that plants allocate to their leaves, stems and roots. Plant dry mass consists of a number of major compounds such as lipids, lignin, N containing organic compounds, hemicellulose, non-structural sugars, organic acids and minerals (Lambers and Poorter 1992). Researchers have been studying variation in dry mass partitioning between leaves and roots (Lambers and Poorter 1992). Plants must distribute dry mass between above and belowground parts to improve their ability to capture light and CO_2 from the aboveground environment, and water and nutrients from soil (Gedro *et al.* 1996; Cao and Ohkubo 1998; Weiner 2004). Results showed that *A. paniculata* adjust their pattern of distributing dry mass between different organs to suit the changing light and N availability. This is consistent with the optimal partitioning theory (McCarthy and Enquist 2007; Kobe *et al.* 2010).

In this study, the most limiting resource was N and the plant organ acquiring this limiting resource was the root. Roots are costly structures for plants to build and maintain. Mechanisms promoting efficient foraging and uptake of limiting soil resources without creating living tissue temper the costs of morphological root foraging in a heterogeneous soil matrix (Jansen *et al.* 2006). At 42 and 56 DAT, depleting N supply in plants with low N rates might have activated genes involved in producing primary metabolites in shoots. Activating these genes changes the metabolic processes in shoots, which involve phytohormones such as auxin and abscisic acid (Maathuis *et al.* 2003). This changing hormonal balance in the root tissue changes the morphology of root systems. Auxin and abscisic acid stimulate plants to transport additional photosynthates or carbohydrates to roots improving their ability to acquire N (Lopez-Bucio *et al.* 2003). Increasing sugar supply to the root affects root morphology through sugar signaling. Sucrose promotes cells to differentiate and mature while hexoses favor cells to divide and expand (Paul and Driscoll 1997). Therefore, depleting N affects primary photosynthesis, sugar metabolism and carbohydrate partitioning between source and sink tissues (Hermans *et al.* 2006). This chain of events might have caused RSR and LMR of *A. paniculata* to change at 42 and 56 DAT.

The size of a plant results from carbon assimilation and respiration, and organ senescence integrated over time. Growth depends not only on carbon-exchange rates of different organs but also on their sizes, morphology and spatial arrangement. This changing pattern of dry mass allocation can affect the growth rate of plants (Poorter 1989; Lambers and Poorter 1992; Shipley 2006; Lambers *et al.* 2008). Relative growth rate measures growth efficiency while NAR indicates the efficiency of leaves in producing dry mass (Poorter and Nagel 2000). Plants must adjust their morphology by an amount and rate that matches the changing light and N availability to maximize growth. This study showed that fluctuating resource availability is probably causing the erratic RGR and NAR trends in *A. paniculata* from initial growth until final harvest at 56 DAT. Plants grew rapidly after they were first side-dressed with N at transplanting indicated by the increasing RGR. Growth continued until 14 DAT, where RGR began to decrease probably because of depleting N in the soil. After plants were side-dressed with N for the third time at 28 DAT, RGR increased exponentially until 42 DAT where growth rate decreased again. Relative growth rate gradually decreased until final harvest at 56 DAT. This phenomenon suggest that plants were actively producing vegetative tissues, which required N in large amounts causing the existing soil N supply to deplete. When N is abundant, growth rate increases. Net assimilation rate also increased when N supply was not limiting, similar to RGR.

If RGR changes during growth, other growth components including NAR, SLA, LAR and/or LMR must also change because RGR is a function of these variables (Lambers and Poorter 1992; Shipley 2000; Lambers *et al.* 2008). This study showed that NAR, SLA, LAR and LMR of *A. paniculata* had changed during growth especially at 42 DAT. Shade and N caused plants to increase total leaf area and leaf dry mass which also increased their SLA and LMR. High SLA and LAR increased their capacity to capture light and enhance productivity. Plants also responded to limiting N supply by increasing LMR and RSR to maximize the ability of roots to take up N from soil. Soil N availability is critical because it frequently limits plant growth (Aerts and Chapin 2000), and fluctuates strongly in many tropical and temperate ecosystems (Lodge *et al.* 1994; Farley and Fitter 1999). Because of this variation in soil N availability, a well-adapted plant must be able to adjust its root system according to timing, rate and amount (Levins 1968). When soil resources are limiting, the ability to alter root systems to maintain viability and growth, and minimize loss of performance is a key aspect of plasticity. Plants could buffer fluctuating resource availability by reducing their growth, which benefits the plants especially in growth during periods of resource scarcity.

This study suggests that *A. paniculata* can adapt to varying levels of shade and N by adjusting its growth and morphology. This phenotypic plasticity might result from altered physiology within the plant.

Studying the physiological changes could add more information on the state of *A. paniculata* during these morphological changes. To conclude, growing under shade and fertilizing with high N rate can enhance growth and productivity of *A. paniculata*. Shading at 40% and fertilizing with 225kg N ha^{-1} can increase dry mass and prevent yield loss, ensuring the quality of *A. paniculata*.

Conflicts of interest

The authors declare there are no conflicts of interest regarding the publication of this article.

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